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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

JANUARY 1945

THE CHEMOTHERAPY OF SYPHILIS*

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SOME years ago I was so misguided as to have joined the long ranks of doctors who had written books on the treatment of syphilis. I was sufficiently bold, eleven years ago, as to entitle my own effort "The *Modern* Treatment of Syphilis" (the italics are new and ironical). Today my own book and the many which preceded it are of historical interest only; and when now the question is asked "How do you treat syphilis?", my answer is "I don't know, but in company with many others, I'm trying to find out."

In an effort to clarify thinking, it has seemed profitable to review the chemotherapy of syphilis over a period of 451 years from the first appearance of the disease in Europe in 1493 until the present day, to be entirely accurate until eight o'clock on the evening of October 12, 1944. Such minute accuracy in point of time is not only desirable but actually necessary, since developments are now so rapid as to change the treatment of syphilis almost from hour to hour. A review of this type is of more than historical interest, since it involves consideration not only of what doctors have done and are now doing, but also of

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why and how these things have been and are being done. It is the "why and how" of the past and present that are important for the future.

Parenthetically, one might believe from the medical literature of the past few years that the very word "chemotherapy" dates from the introduction, only yesterday, of the sulfonamides. Actually, chemotherapy may be said to have begun more than 450 years ago with the disease under consideration tonight, syphilis.

The chemotherapy of syphilis is divisible into three time periods of very unequal length, the first lasting 410 years, from 1493 to 1903; the second of 40 years, from 1903 to 1943; the last, in which the doors of knowledge have as yet opened only a tiny crack for 16 months, from June, 1943, to the present moment. The amount of space devoted in this presentation to these three time periods must be in inverse proportion to the duration of each.

The first 410 years may be dismissed in a few words. When syphilis was first recognized as a new disease by our medical colleagues of the 15th century, its treatment, like the treatment of almost all other diseases, developed on a completely empirical basis. The entire gamut of the Renaissance pharmacopeia was tried, singly and in multiple combinations, ranging from blood letting to hen's dung. Of these, none was successful except mercury, which first came into general use about 1500. This poisonous metal did seem to be successful in healing the open lesions of the disease; though whether it was actually curative was the subject of a 400 year long medical debate, never finally settled until mercury had become obsolete. Given its demonstrated healing powers, however, various methods of its administration were elaborated, and unhappy patients were mercurialized by mouth, through the lungs by inhalation, through the skin by inunction, and later parenterally by intramuscular or intravenous injection. Two facts became evident within a few years; that mercury in excess produced grave and even fatal toxic manifestations; and that to effect the apparent cure of syphilis in an important proportion of cases, it was essential to continue the use of mercury over a very long period, measured in terms of years.

These two facts produced two opposing schools of medical thought, important because of their bearing on subsequent developments. One group of physicians believed that mercury should be given in massive doses, to the point of producing serious toxic symptoms in the patient, from which he was permitted to recover by a rest period without treat-

ment. One single course was demonstrably ineffective. Therefore, many such courses should be given; and the intermittent treatment of syphilis was born. Another group, convinced of the undesirability of adding the dangers of mercurial poisoning to the discomforts and hazards of syphilis, preferred to use less strenuous forms of treatment, with the drug given in small doses without the interruption of rest periods, the analogue of later continuous treatment.

In complete ignorance of the cause of syphilis, of the biology of the disease in man, of immune processes, and of the mechanism of action of mercury, these two schools, one of intermittent and the other of continuous, but both prolonged, treatment systems, flourished side by side for four full centuries, during which the only minor advance was the introduction in the early 19th century of iodine. This drug, given as a halogen iodide, was shown to have healing but definitely not curative effect in syphilis, presumably on the basis of its ability to "dissolve" granulomatous tissue. It came to be used empirically in combination with mercury on the theory that by speeding up the healing of lesions, it enhanced the penetration of mercury into those lesions and thus aided in the destruction of the then unknown agent of the disease.

In the seven-year period, 1903 to 1909, all this was changed. In 1903, syphilis was for the first time transmitted to experimental animals by Metschnikoff and Roux, thus permitting the laboratory study of existing and new methods of treatment. In 1905, the causative agent of syphilis, the *T. pallidum,* was discovered by Schaudinn, thus allowing for a systematic study of the biology of syphilitic infection. In 1907, the blood test for syphilis (now possessing so many modifications as best to be known by the generic term "serologic test for syphilis," abbreviated STS) was developed by Wassermann, Neisser and Bruck, thus immensely widening the opportunities for diagnosis, for immunologic study, and for evaluating the effects of treatment. Finally, in 1909, the modern chemotherapy of syphilis was born through the discovery of salvarsan—606—by Ehrlich and Hata.

The use of arsenic in the treatment of syphilis and the eventual discovery of 606, was no accident, but the result of carefully planned chemical and biologic experimentation over a period of years. The popular name of the drug represents the fact, now often forgotten, that it was the six hundred and sixth preparation which Ehrlich and his co-workers had developed. On the basis of his experimental studies,

Ehrlich originally hoped that syphilis could be "cured" with a single injection of the drug—"therapia magna sterilisans"—and indeed, this was and is possible in experimental animals.

In man, however, and in doses capable of safe employment, the hope was not realized. Patients with early syphilis treated with a single dose of 606 relapsed in high proportions. To meet this disappointment, two expedients were promptly tried—first, to increase the individual dose past the margin of safety advised by Ehrlich, an attempt promptly abandoned not only because of risk to the patient but also because of therapeutic ineffectiveness; second, to give repeated injections of the drug, at first a few, and later as the inefficacy of this was demonstrated, many. The custom of spacing such injections at weekly intervals was based in part on convenience but more importantly on the rate of excretion of 606, which was completed within five to seven days, and in the effort to avoid cumulative toxic effects. Eventually, it was found, in early syphilis, that 606 alone, in any single amount or by repeated doses at any intervals, still provided a relatively high relapse rate; and the minds of clinicians reverted to the possibility of combining arsenic with mercury.

In the meantime, the experimental laboratory had demonstrated that arsenic, as exemplified by 606, was actually spirocheticidal, and that in experimental animals it was curative with a therapeutic ratio of approximately 10, i.e. the lethal dose (MLD) divided by the curative dose (MCD) equalled 10. At the same time, similar laboratory studies had shown that mercury was likewise spirocheticidal, but that its therapeutic ratio was approximately 1, i.e., the dose necessary to cure an infected animal about equalled the killing dose. The beneficial action of mercury in human syphilis, if any, remained and still remains without adequate explanation.

Nevertheless, and in the face of this discouraging experimental evidence concerning mercury, its clinical trial in combination with arsenic quickly demonstrated important advantages, in terms of lowered incidence of relapse and probable eventual cure, over the use of arsenic alone.

Now arose important considerations. Should these two drugs be used separately or simultaneously, continuously or intermittently, and for how long should treatment be continued? On these several points, there was no agreement because of still inadequate knowledge of the

biology of syphilitic infection, the toxicology of the drugs, or their exact mechanism of action. Looking back to the four centuries of experience with mercury, empirical systems of treatment were devised, one of which employed simultaneous courses of salvarsan and mercury separated by rest periods, the other giving courses of each in alternation, treatment being continuous.

Gradually and with increasing clinical and experimental evidence, it became clear that the continuous method of treatment possessed important theoretical and practical advantages. When the drugs were given together by the intermittent system, toxic symptoms from each were more frequent and more severe; the danger of producing a strain of organisms simultaneously drug resistant to both was ever present. The interpolation of rest periods to permit the recovery of the patient from the effects of treatment enhanced the possibility of relapse unless actual "cure" was accomplished by the first course of treatment. Given separately by the continuous system, all these hazards were avoided. The ultimate proof of the pudding lay in final clinical outcome, and by 1926 it had been established that for early syphilis, the continuous method of treatment produced results far superior to those attainable with any other method.

The difficulty lay in the length of time necessary to accomplish these results. Here, by a system of trial and error, and by comparative evaluation of relapse and "cure" in groups of patients given varying amounts of treatment, it was determined that with courses of arsenic injections given at weekly intervals, separated by courses of mercury, 12-18 months of treatment was necessary for early syphilis, 6-12 months for latent syphilis, and varying periods of time, all measured in years, for various types of late syphilis.

This unfortunate prolonged period of treatment, together with the disagreeable, expensive, and sometimes dangerous features of it, posed grave practical difficulties in the way of its mass application. Many patients were too stupid to complete the tedious grind; for all, it was too dangerous, too time-consuming, too expensive; and other expedients were constantly sought.

At first these took the form of attempts to develop arsenical drugs less complicated to administer and less dangerous than 606, which by now was known in this country as arsphenamine. From this search, there came neoarsphenamine, silver arsphenamine, and several others.

In 1925, bismuth was first introduced; and this drug, with a therapeutic index of about 3 to 5, as compared to 10 for arsphenamine and 1 for mercury, gradually came to replace the latter. Still, however, the system of prolonged continuous treatment persisted with alternating courses of some arsenical drug and bismuth (the latter replacing mercury and improving upon its results).

In 1933-1934, two new developments occurred. Tatum and Cooper restudied and re-introduced into the treatment of syphilis an arsenoxide. This substance, the therapeutically active eventual oxidation product of all arsphenamines, had originally been studied but abandoned by Ehrlich as too toxic for human use. In therapeutically effective doses in man and animal, however, it developed that this drug (marketed in this country under the trade names of mapharsen, clorarsen, or phenarsine hydrochloride) was actually less toxic than its parent arsphenamines, that it was excreted from the body with much greater rapidity (from 2 days instead of 5 to 7), and that it was as therapeutically effective as the best of the arsphenamines, 606 itself. Combined with these advantages was simplicity of administration.

Likewise in 1933, Chargin, Leifer, and Hyman in this country and Tzanck in France commenced a re-exploration of the effect of massive doses of arsenic given in a short period of time. Undismayed by the failure of earlier trials of various intensive treatment systems and without preliminary laboratory study, they treated a series of patients with 4-5 grams of neoarsphenamine given by continuous intravenous drip over a five-day period.

Later, because of deaths and a high incidence of serious reactions in the nervous system, mapharsen was substituted for neoarsphenamine, with equally satisfactory clinical results and with a reduction in the incidence of toxic reactions; but still, however, with the frequent occurrence of serious toxic encephalopathies and a mortality rate of approximately 1 to 200 patients treated.

The results obtainable by this intensive method of arsenotherapy given within a five-day period, were equally as good as or better than the best obtainable with prolonged treatment systems requiring 12-18 months for completion; and the method had the inestimable advantage that almost all patients actually completed treatment, although at a considerably increased risk.

This radical innovation quite properly promoted a systematic re-

investigation of the whole problem of metal chemotherapy of syphilis, a laboratory study which should have been carried out many years earlier. Careful experimental study in animals by Eagle and Hogan revealed that the curative dose of mapharsen in early syphilis in rabbits was remarkably constant (5-7 mg./kg. of body weight) regardless of the time interval over which the total dose was given; but that the toxicity of the drug varied in direct relationship to the duration of treatment. Administration by continuous intravenous drip carried no advantage but some actual disadvantages over multiple intravenous injections. Further, the results of mapharsen therapy in animals were improved by the simultaneous administration of bismuth, without significant increase in the toxicity of either drug. Finally, it was shown that the curative dose of mapharsen in early syphilis in man was 3-5 times the curative dose in the rabbit (i.e., 20-35 mg./kg.); and that the same considerations as to toxicity applied in man as in the animal.

This relation of curative to toxic dose permitted the statement that as to arsenic-bismuth chemotherapy of early syphilis, there is no single optimum treatment scheme. Given a required total dose of 20-35 mg./kg. of mapharsen (1200-2100 mg.) administered either by intravenous drip or multiple individual injections, various treatment schemes may be devised with expected and actual mortality rates determined by the duration of treatment. Thus, with all treatment schemes compressed within ten days or less, the mortality is about 1:200. With schemes prolonged for ten to twelve weeks, the mortality from treatment falls to 1:3000 or less. Within these extremes of ten days to twelve weeks, essentially the same clinical results may be achieved, with mortality rates selected as desired to meet the exigencies of the particular patient or the local situation.

Meanwhile, in parallel with these studies of intensive chemotherapy and indeed antedating them, were progressing other studies of the effect of so-called non-specific methods of treatment, especially fever. The clinical acumen of both medieval and modern physicians is challenged by their failure to appreciate the significance of the first record, in the *Autobiography of Benvenuto Cellini*, of the "cure" of his own syphilitic infection by the fever of intercurrent malaria. Not until 1917 did the classical clinical studies of Wagner von Jauregg establish the value of fever (malaria) in the treatment of that most serious manifestation of syphilis, general paresis; further clinical and experimental in-

vestigations have thoroughly established the value of fever therapy, whether from induced malaria or by mechanical means, in almost all forms of neurosyphilis. Indeed, in patients with neurologic damage from syphilis, fever alone is more efficacious than any form of chemotherapy, and there is reason to believe that post-fever chemotherapy does not add much to the results obtained from fever alone. Curiously enough, however, and for reasons not yet clearly known, the value of fever therapy per se is almost entirely limited to neurosyphilis. There is ample clinical and experimental evidence to show that fever alone, in any degree tolerable by man, will not cure early syphilis. However, more or less empirically, there have been numerous clinical attempts, in all stages of syphilis in man, to combine fever therapy with chemotherapy, resulting, in early syphilis, in an effort to compress all treatment into a single day (the highly publicized "one-day cure of syphilis"). The laboratory has again lagged behind the clinic in the study of this important problem; but forced into it by clinical pressure, the experimentalists have now demonstrated (for phenarsine hydrochloride in syphilis) that while the toxicity of the drug is doubled at fever temperatures, its therapeutic efficacy is increased four-fold or better. These toxicity/efficiency ratios during fever appear also to hold for many other drugs, e.g., the sulfonamides.

With any method of use of the arsenic-heavy metal treatment of early syphilis, whether in 12 to 18 months of continuous weekly treatment, in 5 days to twelve weeks of intensive or semi-intensive treatment, or in 1 to 3 days of combined fever-chemotherapy, the results are about the same. These are 80-85 per cent "cure," as measured by prolonged seronegativity of blood and spinal fluid and freedom from early relapse. The late results of adequate prolonged therapy are also known, and comprise about 5 per cent of the development of cardiovascular and neurosyphilis in contrast to the 30-40 per cent expected in the absence of all treatment. With the newer intensive and semi-intensive methods, these late results will not be available for another generation.

Th applicability of the prolonged arsenic-heavy metal systems to the treatment of various types of late syphilis has been fairly well determined after a total period of thirty-five years, as has that of fever therapy, alone or combined with chemotherapy, to neurosyphilis. As to the newer intensive treatment systems, nothing is as yet known in this respect.

Through all the years 1493 to 1943, 450 of them, the questions uppermost in the minds of doctors have been, both in respect first to mercury, later to arsenic-heavy metals; what preparations to give? by what route of administration? alone or in combination with another drug or non-specific method of treatment? continuously or in alternation? and for how long? In their simplest form, all these questions look toward the solution of a problem inherent in all forms of chemotherapy, whether of syphilis or some other disease, the time-dose relationship.

Through all these 450 years, moreover, attempts to solve these problems have proceeded (until quite recently) on an individual rather than on an organized basis. In all countries (until recently) laboratories have thought it sufficient to announce that a given drug or a particular preparation of it was of value in syphilis. How the drug should be used in terms of the above questions was in general left to the clinicians. The latter, working independently of other clinicians and therefore necessarily each on a small scale, tried various systems and combinations of treatment, each trying to persuade the other that his particular plan was best. For the first time, in the late 1920's, the League of Nations and a little later the Cooperative Clinical Group (sponsored by the U. S. Public Health Service) undertook a systematic study of the results of various treatment schemes in early syphilis. The result of this world-wide disorganized study was that as late as June, 1943—only a short 17 months ago—there was still disagreement among doctors as to the best manner of treating even the simplest form of syphilitic infection, the acute early stages of the disease.

Within almost the twinkling of an eye, this picture was even more abruptly changed, in June, 1943, than by the advent of salvarsan, 34 years before. In that month, Mahoney, Arnold, and Harris demonstrated that penicillin was effective in early syphilis in rabbit and man. This drug, for all practical purposes completely non-toxic, appeared to be curative in early syphilis when administered within the brief period of eight days. There was initial promise that a rapid cure of early syphilis had been discovered which was completely safe, lacking all the hazards of the poisons arsenic and bismuth.

Fortunately for penicillin and the advancement of knowledge concerning it, our country was at the moment and still is engaged in a great war. The problem of syphilis, especially early syphilis, is of paramount importance to the Armed Forces; and for the sake of conserving

man power, it is urgent to determine quickly the optimum method of use of this new and revolutionary drug. In place, therefore, of releasing penicillin to all of the syphilis clinics of the country, each of which might pursue its own independent avenues of study, it was clearly obvious that an organized investigation was essential.

This was brought about through the coöperation of the U. S. Army, Navy, and Public Health Service and the Committee on Medical Research of the Office of Scientific Research and Development, under the general auspices of a Penicillin Panel appointed by the Subcommittee on Venereal Diseases, National Research Council. Together with the Army, Navy, and the Public Health Service, some twenty-five civilian clinics were invited to participate in a study of the effect of penicillin in early and late syphilis, the results in early syphilis to be pooled and statistically analyzed as a group.

In addition, the coöperation of four laboratories was enlisted in order to study in experimentally infected animals the various permutations of the time-dose relationship in both early and late syphilis; such other important issues as the effect of penicillin in combination with other chemotherapeutic agents as arsenic and bismuth, or in combination with fever. Both in clinic and laboratory are progressing comparative studies of the several penicillin salts, sodium, calcium, and ammonium; methods of prolonging the action of the drug; and other important pharmacologic investigations.

The urgency of the military situation does not permit, however, that the application of penicillin to the treatment of syphilis in human beings be postponed until all available information can be had from experimental animals. Experimental study must progress simultaneously in man; and is rendered particularly feasible because of the almost complete lack of toxic effects from the use of this drug.

The problems of special importance to the Armed Forces are early, latent, and neurosyphilis, in that order. Most of the clinical emphasis has thus far been placed on the two of these in which results are readily measurable, early and neurosyphilis. The problem of latent syphilis has been relegated to the future for large scale study, primarily because it is essentially a long term investigation, the results of which depend on ultimate clinical outcome after many years of observation.

In early syphilis, all coöperating clinics have agreed to work on assigned treatment schedules devised to investigate the effect of the

several variables of (1) route of administration; (2) the interval between injections; (3) the total duration of treatment; and (4) the total dose of drug. Early effort has been centered on these four factors, in order to determine as rapidly as possible the effect of penicillin alone. The fifth variable, the effect of penicillin in combination with other agents (e.g., arsenic, bismuth, fever) has as yet barely been touched clinically, though it is of course under laboratory study.

The results to date permit a number of general statements. A single injection of penicillin, by any route of administration and in whatever amount, is practically ineffective in early syphilis of man or animal; multiple injections are necessary. The intramuscular route of administration of aqueous or saline solutions of penicillin is certainly as good as, and probably superior to, the technically more difficult intravenous route, whether the latter is given by repeated injections or by continuous drip. With aqueous or saline solutions, the optimum interval between single intramuscular injections ranges from three to six hours, certainly not in excess of the latter. Absorption and excretion of the drug is complete within this time range; methods of prolonging absorption by means of such expedients as oil suspension, implantation of pellets, etc., are not ready for mass experimental study in man. The intrathecal injection of penicillin in patients with early neurosyphilis (e.g., asymptomatic neurosyphilis, acute syphilitic meningitis) is not necessary; although the drug does not penetrate into the cerebrospinal fluid, it does penetrate the diseased tissues of the nervous system. This is almost certainly true also in late neurosyphilis.

With these data in hand, treatment schemes so far employed have been built around the factors of repeated intramuscular injections of aqueous or saline solutions of penicillin given at intervals of every three to six hours day and night. The next problem has been to determine total dose and total duration of treatment—again the perennial time-dose relationship.

In man, the first effort in this direction was to study the comparative effectiveness of four treatment groups, given total doses of penicillin within the 20-fold range of 60,000 to 1,200,000 units divided in 60 intramuscular injections given every three hours day and night for a standard duration of 7½ days. At once, there emerged the fact that within this enormous variation of dose, immediate results were practically identical. Spirochetes disappeared from open lesions within the

time range of six to sixty hours (average about twelve hours); lesions healed with great rapidity; and there was a rapid trend toward reversal of the blood serologic test which proceeded at almost the same rate regardless of dosage. These factors could not be used, therefore, to determine the effectiveness of a given treatment schedule. The only factor which has so far proved of importance is the comparative incidence of relapse, clinical or serologic; and determination of this relapse rate is possible only after the passage of many months of time. It must be repeatedly emphasized, therefore, that the data so far available are based on relatively few cases followed for relatively short time periods. Several thousand patients have been treated, but only a few hundred of them have been followed for six months or longer.

Keeping in mind the preliminary nature of the results, however, it is already indicated that within the range so far studied the relapse rate is in inverse ratio to total dosage; the lower the dose, the higher the relapse rate. With a total dose of 60,000 units given in 7½ days, the indicated eventual early relapse rate is 100 per cent; with 300,000 units, about 75 per cent; with 600,000 units, about 40 per cent; and with 1,200,000 units, about 15-20 per cent. This last rate, 15-20 per cent, is only a little worse than the best that one can accomplish with arsenic-bismuth chemotherapy whether given by intensive, semi-intensive, or prolonged systems; and penicillin holds the inestimable advantage of freedom from all the serious risks of metal chemotherapy.

The next problem is, of course, the effect of much larger doses. Acting on the time-honored lay theory that if one pill is beneficial, two must be better, and four perhaps better still, a large series of cases has been treated with 2,400,000 units in 7½ days, and is now under observation. The time elapsed is too short to permit definitive comparisons, but relapses seem less frequent than with half this dose.

Under simultaneous study is the variable of time. So far, only one comparison is possible. Given in 30 injections in 4 days, a total dose ranging from 600,000 to 2,400,000 units is perhaps less effective than when a similar dose is given in 60 injections in 7½ days. Halving the duration of treatment is therefore not helpful; whether doubling it to e.g. 15 days, while holding the total dose constant improves the situation, is a problem for future study.

Also under current investigation is the effect of lengthening the interval between injections. Here it has already been established that an

interval of 12 hours is unsatisfactory; whether there is any material difference between 3 and 6 hour intervals has not yet been determined.

Finally, in early syphilis, some animal studies and two small pilot groups of patients suggest that penicillin in combination with an arsenoxide (the latter given in eight divided daily injections totalling 5-6 mg./kg. of body weight within 8 days) may be more effective than either drug alone.

The treatment plans so far utilized by the coöperating clinics in early syphilis are shortly to undergo complete revision in order to provide further information as to the several variables involved. It is believed that with this type of organized investigation, optimum methods of use of penicillin in early syphilis can be developed within three to five years, thus obviating the thirty-five years of uncertainty which followed the introduction of arsenic.

So much for uncomplicated early syphilis. Simultaneously, it has been shown that penicillin is apparently completely effective in early syphilis treatment-resistant to arsenic and bismuth; it appears to be of great value in the prevention of congenital syphilis in infants born of recently infected mothers; it produces dramatically favorable effects in early neurosyphilis, asymptomatic or meningeal, in both of which spinal fluid abnormalities improve with even greater rapidity than does the blood serologic test; and it is equally as useful in infantile congenital syphilis as in the acquired disease. (In infants, the total dosage employed in 8-12 days of time has ranged from 20,000 to 60,000 units/kg. of body weight.)

Patients who have relapsed, clinically or serologically, after any dose of penicillin have been re-treated with doses at present regarded as large (i.e., 1,200,000 to 4,000,000 units). So far, no example of penicillin resistance has been encountered, in the sense that relapsing lesions have responded as promptly as did the initial outbreak. It is probable that the relapse rate in re-treated patients will be higher, however, than in patients treated for the original attack, a phenomenon also obvious with metal chemotherapy.

Late syphilis of various types presents a much more complex problem. Here the initial demonstration was that penicillin heals visible gummatous lesions of the skin and mucosae with even greater rapidity than does metal chemotherapy. In interstitial keratitis of congenital syphilis, the results are less spectacular; about 50 per cent of the pa-

tients treated have improved. In neurosyphilis of various types, ranging from asymptomatic neurosyphilis of mild degree to general paresis, little can as yet be said. Some degree of immediate clinical improvement is apparent in most symptomatic cases; but whether these results are as frequent or as complete as after fever therapy is as yet debatable. In most patients with neurosyphilis of any type, there is prompt improvement in the spinal fluid abnormalities, especially in cell count and protein content, and sometimes in complement fixation and colloidal tests as well. It is not yet known whether this clinical and serologic improvement will be maintained.

Dosage schedules in late syphilis are much more difficult to determine than in early syphilis, and many variables have been employed. In general, the major effort of study has been in neurosyphilis; and here, at the moment, four schedules are under trial. These are:

1. Repeated courses of relatively small doses, e.g., 1,000,000 units, separated by rest intervals.

2. Single massive dose courses, ranging from 2,000,000 to 6,000,000 units, given within 8 to 20 or more days. In general, the larger doses are under study in patients with paresis, pre-paresis, or primary optic atrophy; the smaller doses in less serious forms of neurosyphilis.

3. Malaria plus 2,000,000-4,000,000 units of penicillin simultaneously administered, an expedient possible because of the lack of effect of penicillin on malaria. There is some indication that penicillin, like other drugs, is more effective at fever than at normal body temperatures.

4. Malaria followed by penicillin.

The evaluation of penicillin in late syphilis, and optimum methods of its employment, are obviously complicated problems which will require many years of study. Here, as in early syphilis, however, results will be much more quickly available through organized coöperation of many investigators than by isolated individual effort.

One further fact emerges from the use of penicillin in late syphilis of all types. There is evident a trend toward seroreversal of the blood test in a higher proportion of cases than was previously possible from metal chemotherapy. Whether this is necessarily a favorable sign would lead us into a discussion of the phenomenon of seroresistance, for which there is neither time nor space available here.

Reactions from penicillin are negligible except for the Jarisch-Herx-

heimer reaction, consisting usually of fever, sometimes of exacerbation of lesions, within the first twenty-four hours of treatment. The frequency and intensity of this reaction is related to dosage. It occurs in about 75 per cent of patients with early syphilis and about 30 per cent of those with latent and late forms of the disease.

The rapid and safe cure of early syphilis is just around the corner. It is desirable to note, however, that a method of treatment requiring a minimum of ten days of hospitalization for early syphilis is not practical for nation-wide application, especially in rural areas. It is not so much a question of more hospital beds (though this is important) as of the general problem of the economics of antisyphilitic treatment. A method of use of penicillin in ambulatory patients is essential, and is not yet in sight.

As matters stand at present, penicillin is a new and powerful addition to syphilotherapy. How best to use it, alone or in combination with other forms of treatment, is as yet undetermined but is under organized, nation-wide, governmentally sponsored study, from which definitive results may be expected rapidly to emerge. The desirability of continuation of such a scientific approach is exemplified by the historical background of the past 450 years, which I have so briefly reviewed tonight. Perhaps soon again, an answer can be given to the questions of doctor and patient: "How do you treat syphilis?"

THE CHEMOTHERAPY OF GONORRHEA*

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The history of the treatment of the disease, gonorrhea, is as varied and as bizarre as any in medicine. The story spans a period extending from the time of the migration of the tribes of Israel to the immediate present. An almost incredible list of drugs and chemicals has been employed, as well as a variety of biologic preparations; serums, vaccines and filtrates. Their lack of effectiveness in combating the infection may best be attested by the difficulties and problems which were presented by the patient with gonorrhea up to the very recent past. The most remarkable chapters have been written within the last decade during which time the disease has passed from the domain of the local therapist and into the scope of chemotherapy.

In attempting an evaluation of the effectiveness of chemotherapy in gonorrhea, recourse will be taken to the appraisal of the two objectives of such therapy, namely, the curative and the public health or preventive. The former is based upon the facility and completeness with which the disease is brought to arrest and the individual returned to a state of health. The second appraisal takes into consideration the ability of the therapy to reduce mathematically the opportunity for transmission of the infection, thus becoming an instrument in the reduction of the incidence of the disease in a population group.

Not any attempt will be made to review or to comment upon the relative value of the many forms of treatment which have been advocated other than the following generalization. The drugs and chemicals whose effectiveness was dependent upon systemic response, and this may well include the biologic preparations, in a large measure failed to produce consistent curative results. Principal reliance finally centered upon the local application of various antiseptic agents. In the light of the histopathology of the disease, especially as concerns the male ure-

* Presented October 12, 1944 by Dr. J. F. Mahoney at the Seventeenth Graduate Fortnight of The New York Academy of Medicine.

thra, the possibility of even a most active germicide penetrating to the site of the inflammation without affecting adversely the tissue structure, appears to be remote. The duration of the disease and occurrence of complications in many instances reflected an unwise degree of vigor and heroism displayed in the application of local measures. On the whole, the entire concept of local therapy appears, in retrospect, to have been of limited curative value.

The sulfonamide era may be assumed to have had its inception in 1936 when the first of the sulfonamides, sulfanilamide, was made available for study. In the following year the publication of the results of a group of well-conducted studies established beyond a reasonable doubt that the compound was effective in the cure of gonorrhea through systemic or chemotherapeutic action. This conclusion was the first of a series of events, the cumulative effect of which has resulted in an almost complete alteration in the potentialities of the disease as regards its ability to injure the human. The finding also ushered in one of the most dramatic chapters in medicine.

The shortcomings of sulfanilamide therapy were soon apparent. An objectional, but not significant or dangerous, cyanosis accompanied the ingestion of curative amounts of the compound. When used over a prolonged period, or in excessive amounts, definite damage to the blood-forming organs was a possibility. A degree of mental confusion and some interference with muscular coördination rendered the therapy somewhat dangerous for other than hospitalized patients. Of primary importance from the public health standpoint was a tendency to produce symptomless states in which virulent organisms still persisted, thus giving rise to a carrier problem. Although by far the most efficient remedy in gonorrhea which had been produced, the drug rapidly lost favor and ceased to be a factor with the coming of the more adaptable and more effective sulfonamides.

Sulfapyridine was the second of the group to attain usefulness in the therapy of gonorrhea. It was proven to be capable of producing a high cure rate. Its action was prompt and clearcut. However, the use of the drug was accompanied by a disagreeable gastrointestinal toxicity which also restricted its use to patients under close supervision. This compound, too, rapidly passed from favor when the most important member of the group, sulfathiazole, was developed. This last drug displayed a remarkable freedom from the production of toxic reactions

when used in a conservative manner and was credited with producing a cure rate approximating 85 per cent under favorable conditions. Sulfathiazole has been generally used by clinicians, and almost exclusively by health organizations, in the management of the disease and was not replaced in popularity by sulfadiazine or others of the more recent sulfonamides.

In an excellent review Henry[1] summarizes current thought on the subject of the mode of action of the sulfonamides. There is general agreement that the present concepts undoubtedly will change as more complete knowledge becomes available. The consensus at the moment favors the view that this group of compounds inhibits the growth of cells and that this inhibition involves not only the bacterial cell but the tissue cells as well. The degree of inhibition seems to be dependent upon the concentration of the compound, the type and the environment of the cell.

Evidence available at this time supports the view that the bacteriostatic effect of the compounds is due to a direct inhibitory influence upon one or more enzymes. That the action is reversible may be demonstrated by the restoration of cellular activity after washing out of the sulfonamide compound.

One group of investigators believes that the sulfonamides interfere with the enzymes which catalyze anabolic and catabolic reactions within the cells. The antagonism of para-aminobenzoic acid to the action of a sulfonamide is explained on the assumption that an excess of para-aminobenzoic acid effectively minimizes the combination of the sulfonamides with the enzymes necessary in anabolic reactions which utilize para-aminobenzoic acid as a substrate. However, there appears to be support for the belief that the sulfonamides produce primary inhibition of one or more enzymes concerned with oxidation-reduction and thus interfere with cellular division.

Dorfman, et al,[2] point out that the bacteriostatic effect of sulfapyridine and sulfathiazole appears to be caused by the inhibition of reactions other than those which are influenced by sulfanilamide. They conclude that the action of the sulfonamides may be explained on the basis of differential interference among several enzyme reactions. Such a selective capability appears to be indicated by clinical experience.

Early experience with sulfonamide therapy in gonorrhea demonstrated that a proportion of patients, approximating 15 per cent with

sulfathiazole, failed to attain curative response following a routine of treatment which proved to be effective in a large majority of instances. The reasons usually put forth to explain these failures were (a) the chance encountering of a strain of N. gonorrhoeae whose biology differed from the average to the extent of being resistant naturally to chemotherapy and (b) that the failure patients represented a deficiency, or absence, of some host factor which was essential to the effective action of the compound. A brief discussion of these concepts may be of interest.

At the outset of the work it was necessary to ascribe any resistant characteristics to natural phenomena, as not any of the strains had been exposed previously to the sulfonamides with sufficient frequency to stimulate a biologic change of the degree manifested by the resistant strains. Hence, it must be assumed that the ability to survive was the result of experience with destructive agents other than the sulfonamide type of compounds. Just why only a relatively few strains should have had this experience is not clear.

It was not until recently that a specific contribution to the subject of strain resistance could be made. In utilizing this reference and material, recourse will be taken to an unpublished study, the results of which probably will be made available in the near future.

Under conditions of incarceration male volunteers were exposed to infection through the urethral instillation of culture material containing virulent gonococci. The percentage capabilities of various strains to produce clinical disease does not enter into present considerations. Of interest is the evidence which points to the presence of strain resistance and to the tendency of an individual strain to follow a characteristic behavior pattern as regards its response to sulfonamide therapy.

A strain of N. gonorrhoeae isolated from a prostitute who had failed to attain clinical cure through repeated courses of sulfonamide therapy was purified and utilized as inoculum in the exposure of a group of 19 volunteers. Of this group 10 developed clinical gonorrhea confirmed by laboratory findings. All of the patients failed to respond to sulfathiazole therapy in a satisfactory manner and were brought eventually to clinical and bacteriological cure through the use of penicillin.

The converse of this was also studied. A strain of N. gonorrhoeae isolated from a male patient who had responded in a satisfactory man-

ner to sulfathiazole therapy produced clinical disease in 25 volunteers. All of this group attained prompt relief of symptoms and negative bacteriological findings following the use of sulfathiazole.

Although the material is not large the observations were very precise and pointed toward the ability of strains to retain susceptible and resistant characteristics through the small number of passages observed.

The second concept, that involving host factors, may be dismissed rather hurriedly because of the lack of exact information upon the subject. Early in his studies in chemotherapy Ehrlich postulated that a degree of immunological response was a prerequisite to the efficient action of any chemotherapeutic compound. This thesis must have been based upon philosophic grounds as the procedures which are used to measure the usual immunological responses fail to show any difference in the economy of patients whose infections responded to the sulfonamides and those which failed. Neither are clinical means available which serve to identify or measure the host factors which serve to enhance the action of the chemotherapeutic agent.

That host factors are of importance is demonstrated by the results which are obtained in patients who contract the disease from a single source and hence should be considered as having been infected by the same strain. In several instances of record, one patient has been known to attain a prompt cure while the second has proven resistant to sulfonamide treatment. The identification of these host factors and their stimulation or alteration to assist better in the promotion of drug action still constitutes one of the most important problems in chemotherapy.

In attempting an estimate of the over-all benefit which has been derived from sulfonamide therapy the published cure rates probably present a conflicting picture. In studies carried out under hospitalized conditions wherein the patient actually receives a stipulated amount of the drug over the stated period of time and where the post-treatment observation is carried out in a precise manner and the patient is maintained free from a reasonable possibility of reinfection, the cure rates have approximated 80 per cent. In studies in which outpatient material was employed lower rates have, as a rule, been produced. This decline in effectiveness is to be expected as the actual carrying out of the outpatient treatment schedules depends entirely upon the type of coöperation which the patient contributes. Basing conclusions upon the findings

of studies wherein the most essential factor, that of total ingestion of drug, is at the discretion of the patient will always be hazardous.

Because of the lack of an accurate statistical backlog it becomes impossible to follow the trends of gonorrhea in anything like a precise manner. The peace time levels are not known and probably will not be known in the future. Reflected in the statistical patterns, however, are evidences of marked increases in prevalence during and following periods of national conflict. The upstrokes due to World War I are clearly defined. At the present the gonorrhea situation appears to be entirely favorable. The anticipated increases in rates have not, up to this time, taken place. Such reports as are available from military organizations located within the continental limits of the United States are appreciably below those of the previous conflict. These rates are generally in the neighborhood of 30 per 1000 per year for the venereal group of diseases with gonorrhea contributing the usual two-thirds.

Any decline in the gonorrhea rate, if it be the result of a man-made effort and not a natural cycle in the disease itself, must be ascribed to the efforts which have been directed toward the control of the disease in recent years and which have been augmented greatly during the war period. Basically, these efforts have been dependent completely upon sulfonamide therapy until quite recently when penicillin has been added. The ability of the therapy to curtail the period of infectiousness, thereby markedly reducing the mathematical opportunity for transmission in a fair proportion of patients, may be responsible for placing in motion a geometrically progressive decline in incidence. Such a decline may be expected to continue unless some unforeseen and unsuspected factors arise to impede the working of an accepted law of epidemiology.

Before a true estimate of the real value of sulfonamide therapy could have been established the antibiotic substance, penicillin, was found to possess an almost miraculous efficacy in checking gonococcic infections.

The developmental work in penicillin therapy was carried out largely on the initiation of the National Research Council. The early studies rapidly demonstrated the effectiveness of the product and subsequent efforts were directed toward the finding of the optimal dose-time ratio.

In one of the early studies[3] a treatment period of 48 hours was

employed. Because of the frequency of dosage and the use of intramuscular injections hospitalization of the patients became necessary. The opposite extreme was reached in a very recent study in which Van Slyke[4] and others utilized treatment periods of 4 and 8 hours, respectively, giving only two injections, each of 80,000 units of penicillin. The cure rates attainable with this routine were high—92 per cent and 96 per cent. The inference is inescapable that a routine of this kind employed in clinics or in office practice would become a most effective instrument in a mass attack upon the disease.

In the matter of dosage, a wide range of effectiveness is probably demonstrable in different individuals. Excellent results have been observed in patients receiving as low as 50,000 units and failures at 200,000 units. This, again, may be an expression of the presence or absence of host factors. A safe and efficient level of treatment appears to involve, roughly, a total of 120,000 units given by intramuscular injection, 20,000 units at 3 hour intervals over a treatment period of 15 hours. The amount of drug may be doubled or the duration of treatment reduced appreciably without greatly altering the cure rate.

In a recent article, Sternberg and Turner[5] report upon Army material. The work is important because the generally satisfactory results produced represent the pooled experiences of a group of clinicians rather than those of an individual, and also because of the fact that it was not necessary to take recourse to any type of therapy other than penicillin to effect a cure in approximately 1800 patients included in the study. A similar experience has been reported by others.

Not anything is known of the biologic mechanisms, or the reasons therefor, which produced in one of many species of mold the capacity for the destruction of bacterial forms of life, among them, the gonococcus. The chemical nature of the substance is unknown or unrevealed. Its mode of action, like that of the sulfonamides, is ascribed to the power of blocking enzymatic action which is essential to the economy of the bacterial cell. In contradistinction to the sulfonamides is the fact that normal tissue cells are not involved in the action. The real importance of penicillin lies not so much in its ability to destroy certain pathogens but rather to the impetus which its use has given to investigative work in the field of antibiotics. The concept that antibiotics may be effective chemotherapeutic agents is itself old. Dubos,[6] in the development of gramicidin, made a most important contribution.

Research of the future will be directed toward the discovery of other forms of life which possess the capacity to produce substances which may be utilized in the destruction of pathogenic organisms.

In the recent literature[7] some alarm has been expressed as to the role which will be assumed by gonorrhea in the future. The prognostications include the production of symptomless carrier states and of drug resistant strains which will move unchecked through a population group in the proportion of an epidemic. In this light, the sulfonamide compounds will come to be looked upon as harbingers of evil and precursors of calamity to be censured and condemned for promoting a relatively inocuous disease to a position of direful proportions.

In the experimental study previously referred to a total of 82 infections was produced and treated with penicillin and/or sulfathiazole. All were closelv followed through prolonged post-treatment periods with careful clinical and cultural observations. Not any of this group was found to harbor the N. gonorrhoeae beyond the eighth (8) day after the cessation of clinical symptoms. This evidence, for what it may be worth, points away from any alarming tendencies toward the widespread development of carriers and the lack of faith in the completeness of cure.

Conversely, if these therapies continue to curtail the duration of the infectious period of the disease in a large proportion of instances, then surely the exponent of public health may look forward with confidence to a future world relatively free from the not entirely pleasant influence of gonorrhea.

It would be wrong to suggest that eradication might be well within the range of possibility, as the complete destruction of any species in nature runs counter to all biologic law. In all probability, the disease will always be with the human race but its capacity as a producer of human distress and suffering may be reduced to a minimum through the wise application of the knowledge which is available at the moment.

Conclusion

By way of conclusion, if the incidence of the disease as estimated at the present can be induced to remain at its present level for the duration of the war, an appreciable reduction may be anticipated when those at present living under military conditions again assume a more rational mode of existence. When to this influence upon the side of

the defense is added the most dramatic therapeutic agent ever placed at the disposal of the medical profession, with its recognized ability to curtail the infectious period to a matter of hours, then surely the incidence of this disease must further decline to a point where, everything else being equal, the disease should not be an important source of ill health to the human race and its position as a public health problem should have largely passed to a more worthy candidate.

REFERENCES

1. Henry, R. J. Mode of action of sulfonamides, *Bact. Rev.*, 1943, *7*:175.
2. Dorfman, A. *et al.* Inhibition of respiration of dysentery bacilli by sulfapyridine, *Proc. Soc. Exper. Biol. & Med.*, 1940, *43*:750.
3. Mahoney, J. F., Ferguson, C., Buchholtz, M. and Van Slyke, C. J. Use of penicillin sodium in the treatment of sulfonamide-resistant gonorrhea in man, *Am. J. Syph., Gonor. & Ven. Dis.*, 1943, *27*:525.
4. Van Slyke, C. J. and Steinberg, S. Outpatient penicillin treatment of gonococcic infections in males, *Ven. Dis. Inform.*, 1944, *25*:229.
5. Sternberg, T. H. and Turner, T. B. Treatment of sulfonamide resistant gonorrhea with penicillin sodium, *J.A.M.A.*, 1944, *126*:157.
6. Dubos, R. J. Studies on a bactericidal agent extracted from a soil bacillus, *J. Exper. Med.*, 1939, *70*:1.
7. Pelouze, P. S. Gonorrhea: the epidemic we face, *Ven. Dis. Inform.*, 1944, *25*:76.

THE MODE OF ACTION OF CHEMOTHERAPEUTIC AGENTS*

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THERE are, theoretically, several independent avenues of approach to the problem of the chemotherapy of infectious diseases. One may conceive, for example, of the existence of chemical substances endowed with such physiological activity that they hasten recovery by stimulating the normal defense mechanisms of the body. There may be, on the other hand, other types of chemical agents capable of exerting a beneficial effect on the course of the disease by decreasing its toxic manifestations, either by combining with and neutralizing the toxic constituents and products of the parasite, or by rendering the host cells resistant to these poisons. In practice, however, the only significant results of chemotherapy to date have been obtained with substances which are capable of killing, or of inhibiting the growth of, the infectious agent in the invaded tissues of the host. The present discussion will be limited to an analysis of the mechanisms by which antiseptics and chemotherapeutic agents interfere with the living processes of bacterial cells.

The Receptor Theory. The first step in the action of many if not all antimicrobial agents is the fixation of the agent upon some constituent of the susceptible cell. This hypothesis was first clearly formulated by Paul Ehrlich, who believed that antiseptics and chemotherapeutic agents exert their action by combining with certain chemically reactive groups of the cell. He called these hypothetical cellular structures "receptors." Differential susceptibilities to the different growth inhibitors could thus be explained by postulating the existence in the susceptible cell of a sufficient variety of receptors concerned in essential metabolic functions.

For a long time, the inadequacy of the biochemical knowledge concerning cellular structure and metabolism prevented any definition of the nature of the receptors in accurate chemical terms. Ehrlich him-

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self, however, had suggested that the arsenicals owe their physiological activity to their affinity for reduced thiol groups. Evidence has continued to accumulate that the cellular–SH groups are indeed the "receptors" which fix the arsenicals upon the susceptible organisms, and furthermore, it is also believed that the antimicrobial activity of the mercurials is due in part at least to the same type of chemical activity.

Further progress in the formulation of the receptor theory came from the analysis of the mode of action of the antibacterial dyes. Thus, the basic dyes (tryphenylmethane and acridine compounds in particular) appear to owe their biological activity to their basic ions, which form feebly-ionized compounds with the acidic groups of bacteria. Conversely, the acidic ions of the acid dyes (e.g., acid fuchsin) are assumed to react with the basic groups of bacteria giving rise to an ordinary type of double decomposition.

The same general type of acid-base reactions appear to constitute one of the primary steps in the action of other kinds of acidic and basic antiseptics, such as the synthetic soaps known under the name of anionic and cationic detergents. Although it is certain that these surface active compounds owe much of their bactericidal power to their physical properties, it is likely that they first unite with the bacterial cell through their acidic and basic groups respectively. It is worth mentioning at this time that the phenolic compounds have also been claimed to owe their antibacterial activity to a reaction between their acidic hydroxyl groups and some basic cellular components. Since reactions which occur slowly in the bulk phase may occur rapidly when the reactants are oriented in a surface layer, and since phenolic compounds are surface active and therefore orient themselves at the cell surface, the reactivity of the phenolic hydroxy groups can become very great in biological reactions. Even in the test tube, phenols can precipitate amines provided one reactant is of sufficient molecular weight or carries a lipophilic constituent. It is not unlikely, therefore, that phenols combine with the amino groups of bacteria. On this basis, phenolic compounds should be considered as anionic (acidic) antiseptics, a theory which may account for the fact that they are most effective at acid reactions.

The further evolution of the theories of antisepsis has been parallel to the evolution of the theories of enzyme chemistry. With the symbol of lock and key relationship, E. Fisher introduced forcefully, as P.

Ehrlich had done with the theory of specific receptors, the concept that the structural configuration of the reacting molecules conditions the reaction between enzymes and substrates. This theory accounts for the extraordinary specificity of many biological phenomena. It explains in particular the phenomena of specific inhibition which have come to play such an important part in biochemistry, pharmacology, immunology, etc. Thus, in many cases, a given enzymatic reaction can be inhibited or blocked by substances which, because of the similarity of their molecular structure with that of the normal substrate, can react and combine with the enzyme, although they are not transformed chemically by it. Many examples of this type of reaction can be selected from the field of bacterial chemistry. For instance, succinic dehydrogenase is inhibited by compounds containing the group—CH_2COOH, notably by malonic acid, even though this compound is not "activated" by the enzyme. It has been shown that this enzymatic inhibition can result in retardation or inhibition of growth when malonate is added to a bacterial culture proliferating in a medium containing succinic acid as the sole source of carbon. By inhibiting the oxidation of the succinate, malonate deprives the organism of its source of energy. Although malonate acts as an inhibitor of bacterial growth in this system, it is not a cell poison, for it does not act as an inhibitor in systems where a preliminary oxidation of succinate is not necessary to provide the bacteria with carbon for synthesis.

The theory of competitive inhibition has found its most spectacular expression in the discovery that p-aminobenzoic acid can neutralize the bacteriostatic action of the sulfonamides. Sulfanilamide and p-aminobenzoic acid are closely related structurally, differing only in the fact that the sulfonic group of the former is replaced by a carboxyl group in the latter; this relation suggested that the sulfonamides owe their antibacterial effect to their ability to compete, by virtue of similarity in chemical structure, with p-aminobenzoic acid in some essential metabolic reaction. It is not our purpose to examine whether this theory of "competitive inhibition" accounts completely for the mechanism of action of these drugs. It is worth mentioning, however, that there are facts which indicate the occurrence, during bacteriostasis, of a type of union between the aeromatic amino group of the sulfonamide and some structure in the susceptible bacterial cell. Thus, when suspensions of *E. coli* are treated with dilute solutions of p-aminobenzoic acid, sulfanil-

amide, and related compounds, it is found that all the chemotherapeutically active sulfonamides have an effect on the electrokinetic mobility of the organism resembling that produced by the aminobenzoic acid, whereas the effect is quite different in the case of the inactive substances related to sulfanilamide. These facts suggest that the active drugs behave like p-aminobenzoic acid at the bacterial surface and that the association of the drug with the organism is a function of the aeromatic amino group.

Bacteriostatic versus Bactericidal Effect. It is to be expected that this initial primary reaction which takes place between the growth inhibitor and the homologous substrate (receptor) should in some cases be reversible under the proper conditions. For example, modifications of the acid-base conditions in the environment, removal from the medium of any excess of the antibacterial agent, addition of substances exhibiting a great affinity for it, should facilitate the dissociation of the complex formed between the inhibitor and the cellular substrate, and theoretically should restore the cell to a condition where growth again takes place.

Indeed, it has been possible in many cases to reverse the action of antiseptics commonly assumed to exert a bactericidal action. Cultures inhibited in their growth by the presence of a basic dye in a medium at slightly alkaline reaction can be caused to grow out by rendering the medium more acidic, thus decreasing the affinity of the dye for the acidic constituents of the cell. Bacteria "killed" with mercury can be caused to multiply again following treatment with soluble reduced sulfur compounds (H_2S, glutathione, thiolactic acid, cysteine, etc.) which exhibit great affinity for the metal. The phenomenon can be demonstrated with particular ease in the case of bacterial spores which, probably on account of their slow metabolism, can regain their viability after prolonged exposure to mercury when treated with sulfhydryl compounds. Another remarkable illustration of reversibility is found in the inhibition of growth of yeast and of *Lactobacillus casei* by a crystalline basic protein extracted from wheat. Although treatment of these microorganisms with the protein renders them unable to multiply, growth can be reinitiated by the addition to the system of different phosphatides. Since the basic protein is known to occur in wheat in combination with a phosphatide, it is possible that the reversal of antimicrobial action by the phosphatide is due to the formation of a lipoprotein which

helps in dissociating the basic protein from the cell.

According to these views, the bactericidal character of an agent depends upon its ability to form with vital groups in the bacterium non-dissociable complexes. This requirement may account for the fact that surface activity and high molecular weight, two properties which favor the formation of insoluble or feebly ionized complexes, are so commonly found in bactericidal agents.

The ultimate effect, bacteriostatic or bactericidal, of a given agent is conditioned by a great variety of environmental factors among which can be mentioned the composition of the medium, its reaction, the temperature at which the test is carried out, etc. Of obvious importance is the nature of the microörganism involved. Thus, it is a matter of common observation that many agents are much more bactericidal for pneumococci than for streptococci. The nature of the microörganism influences the outcome of the antibacterial test, not only by determining the strength and stability of the combination between inhibitor and susceptible cellular substrate, but also because, during bacteriostasis, there occurs within the cell certain secondary changes which result in irreversible alterations and therefore, in death. The ease with which pneumococci undergo autolysis as soon as the medium becomes unfavorable for growth and multiplication accounts, in part at least, for the fact that many agents which are only bacteriostatic for other organisms exert upon pneumococci a bactericidal effect. The occurrence of secondary irreversible alterations during bacteriostasis may also explain the observation that the bacteriostatic effect of the sulfonamides can be converted into a bactericidal effect by raising the temperature at which the reaction is taking place from 37°C. to 40°C. It is likely that, at the higher temperature, the rate of the catabolic reactions is increased while the compensatory anabolic reactions are inhibited by the drug. The more rapid exhaustion of some reserve substance, or the destruction of some essential structure, may be the direct cause of the bactericidal effect.

It appears, therefore, that the difference between bacteriostatic and bactericidal effect is often of a quantitative rather than of a qualitative nature. It depends upon factors which affect the rate at which irreversible alterations go on within the inhibited cell; it is conditioned by the firmness of the combination between the toxic agent and its "receptor" in the susceptible cell, and by the ease with which the com-

bination can be dissociated.

The Effect of Chemotherapeutic Agents on Bacterial Metabolism. The statement that a given antibacterial agent combines with a certain type of chemical group in the susceptible cell is not sufficient to define the cell structure and function with which it interferes. Thus, it is true that arsenicals and mercurials can combine with reduced thiol groups; these groups, however, are so widely distributed in the cell in the form of cysteine, glutathione, proteins, etc. and play a part in so many different metabolic events that the primary effect of the drugs on cellular structure or processes is not defined thereby. Similarly, the statement that basic antiseptics become attached to the cell at some acidic group does not describe the nature and function of the cellular groups which are affected.

As is well known, the similarity in molecular structure between sulfanilamide and its inhibitor p-aminobenzoic acid, has led to the formulation of the most widely quoted hypothesis attempting to account for the mechanism of drug action. It has been postulated that, in this case, the bacteriostatic action is due to a competition of the drug with p-aminobenzoic acid at some vital stage of metabolism in which the latter substance is concerned. Although there is as yet no direct convincing proof of the validity of this hypothesis, some evidence for it has come from the subsequent demonstration that p-aminobenzoic is indeed an essential growth factor or metabolite for many species. Moreover, indirect support for the theory of competitive inhibition has come from the fact that, by modifying the molecular structure of substances concerned in normal metabolism, it has been possible to synthesize a great variety of compounds which interfere with bacterial growth. Among many other examples, it is sufficient to mention pyridine-3-sulfonic acid, amino-sulfonic acids, thiopanic acid (pantoyltaurine), etc. which bear to nicotinic acid, aminocarboxylic acids, pantothenic acid, etc. the same relation that sulfanilamide does to p-aminobenzoic acid. All these sulfonic analogues of essential growth factors inhibit bacterial growth, and in all cases the inhibition of growth is reversed by the addition to the system of an excess of the corresponding normal carboxylic metabolite. Of special interest is the fact that one of these sulfonic compounds, thiopanic acid (pantoylthaurine) is capable under the proper conditions of behaving as a chemotherapeutic agent against streptococcus infec-

tion, and that this protective effect is neutralized by pantothenic acid.

The increase in knowledge of the intimate processes of cellular metabolism has encouraged the investigation of the mechanism of drug action by the classical methods of metabolic chemistry. Many attempts are being made to determine, not only the effect of antiseptica and chemotherapeutic agents on intermediate metabolism, but also to identify more specifically the cellular catalysts and processes which are affected during inhibition. It must be emphasized, however, that there are few agents if any which are so specific in their action that they affect only one cellular structure or function; sulfanilamide for example, inhibits the enzyme carbonic anhydrase, but this fact is unrelated to the mechanism of its action on bacteria. In other words the discovery that a given antiseptic inactivates a certain enzyme or metabolic process is no evidence that this particular reaction is the one which determines the bacteriostatic or bactericidal effect. It is probably this lack of specificity, this multiplicity of biochemical activities exhibited by all antibacterial agents, which has prevented thus far the elucidation of the primary reaction which results in inhibition of growth or in death.

Antiseptic versus Chemotherapeutic Agents. Granted these fundamental difficulties of interpretation, the analysis of the effect of antibacterial agents on the metabolism of susceptible cells has suggested a few generalizations concerning the characters which differentiate the ordinary antiseptics from the few substances which retain their activity *in vivo* and which can therefore, classify as chemotherapeutic agents. Most common antiseptics interrupt immediately and irreversibly the metabolism of living cells, whether it be measured in terms of oxygen consumption, production of carbon dioxide, production of acid, luminescence or other biological manifestation. In fact, measurement of the inhibition of these metabolic events has often been suggested as a quantitative method for the determination of antiseptic action and of the comparative activities of various agents. Let us consider on the other hand what little is known of the effect on metabolism of some of the few agents which have been found to retain their activity *in vivo*. There is still much argument concerning the intimate mechanism of the action of sulfonamides. It is certain, however, that these drugs do not completely interrupt cellular respiration; bacterial cells inhibited in their growth continue to metabolize even in the presence

of an excess of sulfonamides. Although no quantitative data have yet been published concerning the effect of penicillin on the metabolism of susceptible cells, it has been clearly stated that this drug does not affect the respiration of staphylococci. The comparative effect of gramicidin and tyrocidine is illuminating with reference to this problem. Both these substances are produced by cultures of *Bacillus brevis*, both are polypeptides in nature, both exhibit antibacterial activity. Tyrocidine is toxic not only for bacteria but also for all types of living cells so far tested and is therefore entirely ineffective *in vitro;* gramicidin, on the contrary, although toxic for spermatozoa and erythrocytes, is inactive against many other types of tissue cells, and among bacteria, affects chiefly the Gram-positive species. Interesting enough tyrocidine, like other common antiseptics, completely and irreversibly inhibits cellular metabolism, whereas gramicidin permits the maintenance, even though in a modified form, of oxygen consumption, of carbon dioxide and acid production, and of several other metabolic functions of even the most susceptible cells.

It appears, therefore, that whereas the typical antiseptic behaves as a gross protoplasmic poison, destroying the general metabolic and especially the catabolic cellular mechanisms, most chemotherapeutic agents have on the contrary, a very selective effect on some specific metabolic steps. The nature of the specific steps inhibited will undoubtedly vary from one chemotherapeutic agent to another. There are, for instance, observations which suggest that the primary effect of the sulfonamides is on some synthetic, anabolic, process. In the case of penicillin, it is reported that the drug does not in reality interrupt growth of the inhibited cell but prevents cellular division, with the frequent production of giant forms. Gramicidin appears to affect an energy-using process which would normally allow carbohydrate and phosphate storage. In other words, inhibition of growth can result not only from interference with the normal phenomena of respiration and nutrition, but also from the interruption of some process of synthesis, or of some step in cellular division. Since most of the knowledge of cellular metabolism available at the present time is limited to the catabolic processes which liberate energy, it is possible that no complete understanding of the mechanism of drug action can be reached until we know more of the methods by which the cell utilizes for growth and division the energy which it obtains from nutrition and respiration.

General Remarks. Although the analysis of the phenomena of antisepsis has failed so far to reveal the precise nature of their mechanism, it has yielded information which permits a more rational statement of some of the practical problems of chemotherapy.

The fact, for example, that many agents which are extremely active in artificial media lose much of their antibacterial activity *in vivo* or in the presence of animal tissues or fluids can be analyzed in terms of those factors which have been shown to affect the reaction between cell and antiseptic. Thus, p-aminobenzoic acid and other substances which reverse the action of sulfonamides are produced by many cells and released during tissue breakdown, and undoubtedly play a part in rendering these drugs less active in the presence of exudates. This subject has been discussed so often in recent times that it need not be restated. There is another group of inhibitors of antimicrobial agents which deserve some mention at the present time. It has been observed that several types of phospholipids and a few other surface active substances are capable of protecting living cells against the lethal effect of the subsequent addition of many toxic agents, a fact of practical importance since phospholipids are commonly present in biological materials. It is possible that these compounds form inert complexes with certain basic antiseptics, also that they can exert a protective action by becoming absorbed on the cell surface, perhaps at the very sites at which the toxic agents would otherwise become absorbed.

There is still another type of substances which can inhibit antiseptics and chemotherapeutic agents, especially at the site of infected wounds and burns. We have emphasized earlier in this discussion that many of the antibacterial agents become attached to the parasitic cell through a combination of their basic groups with acidic cellular radicals. These same basic groups of the antiseptics also, probably, combine with the acidic groups exposed at the surface of the tissues, for example those of chondroitin sulphuric acid, the carboxylic groups of various mucoids and of hyaluronic acid, the phosphoric group of nucleic acids, etc. All these acidic groups are present in complexes of high molecular weight, even of colloidial dimension. They may form feebly-ionized complexes with the basic antiseptics and by binding them at the tissue surface, they may render them useless from the antibacterial point of view.

Another fact worth considering in analyzing the reasons why so

many antibacterial agents are ineffective on infected wounds or burns is that the reaction of these tissues is often more acidic than that of the blood. It is a known fact, on the other hand, that most antibacterial agents become less active as the pH is lowered. One may wonder therefore whether the problem of infected wounds and burns will not demand that attention be directed toward a class of substances exhibiting an optimum zone of activity slightly on the acid side.

It has been pointed out that the common antiseptics and the chemotherapeutic agents seem to differ profoundly with reference to the effect which they exert on cellular metabolism. In contrast with general protoplasmic poisons, chemotherapeutic agents exhibit selectivity not only with reference to the type of cells which they affect but also with reference to the metabolic systems or structural constituents with which they react. This problem is not only of theoretical interest, but is also of immediate practical importance. Because of the cost and the labor involved in animal experimentation, many workers have attempted to develop short cut "screening" methods for the rapid evaluation *in vitro* of new antimicrobial agents. These *in vitro* methods often make use of inhibition of metabolism as measured by oxygen uptake, production of carbon dioxide or of acid, luminescence, etc. It is important to realize that, although these techniques would have been successful in detecting the gross protoplasmic poisons, they would have failed to recognize many substances of chemotherapeutic activity; sulfonamides would have been discarded as agents of little metabolic activity; penicillin would have been neglected in favor of the countless other phenolic compounds and quinones produced by molds, actinomycetes and bacteria; tyrocidine would have been selected in preference to gramicidin. The need for *in vitro* screening methods in the search for new chemotherapeutic agents is evident. It is clear, however, that none of the *in vitro* metabolic methods available at the present time are satisfactory. The development of new and adequate assay techniques, as well as the complete understanding of the intimate mode of action of chemotherapeutic agents, may have to await increased knowledge of the reactions concerned in anabolic metabolism and in the processes of growth and cellular division.

CHEMOPROPHYLAXIS OF STREPTOCOCCUS DISEASE*

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CHEMOPROPHYLAXIS is a procedure involving the administration of drugs for the purpose of preventing certain infectious diseases in individuals or groups of individuals. The use of this procedure may be indicated when the probability of acquiring preventable infection is sufficiently great and when the hazards of such infection are of such magnitude as to justify the cost. In time of war the hazards of infection in military personnel are evaluated in terms of interference with the military mission, incidence of crippling disease and death rate. The military need for mass chemoprophylaxis which has been successfully met is the prevention or disruption of the training program. The cost of chemoprophylaxis may be stated in terms of the price of the drug, the time of the personnel necessary to supervise the administration and evaluate the results, the difficulties in discipline, and the frequency and seriousness of untoward reactions. Chemoprophylaxis was first given a place in clinical practice for the prevention of streptococcus disease and recurrence of rheumatic fever in individuals who had had previous attacks of rheumatic fever. This contribution was made independently and simultaneously by Doctors Caroline Thomas[1] and Alvin Coburn.[2] Sulfonamide prophylaxis was later used most effectively in the Army in prevention of meningitis[3] and, on a small scale, to stop an epidemic of scarlet fever.[4] The initiation of large scale chemoprophylaxis for streptococcus disease in the armed forces was again due to the interest of two officers in rheumatic fever—Commander Alvin Coburn[5] of the Navy, and Colonel Paul Holbrook[6] of the Army Air Forces, who independently made use of chemoprophylaxis for the purpose of pre-

* Presented by General Morgan, 10 October 1944, at the Graduate Fortnight of The New York Academy of Medicine.

venting rheumatic fever in military personnel. Striking benefits of mass chemoprophylaxis beyond the effects on obvious streptococcus syndromes were observed in both programs. In this discussion we will deal with some of the problems and lessons of mass chemoprophylaxis as observed in the Navy and Army Air Forces and then see to what extent they may be applied to the problems of individuals.

Technique. All the large scale programs of chemoprophylaxis have used sulfadiazine. There is clear evidence that daily administration is essential and that one dose a day given in the evening is highly satisfactory. One gram daily has been the most satisfactory dose. Regular administration of any drug daily to well people requires regimentation and organization. Responsibility for actual administration rests upon the officers in command of the military organizations. Adequate safeguards must be set up to see that cheating does not occur.

The Benefits of Mass Chemoprophylaxis. The benefits resulting from different programs of mass chemoprophylaxis carried out in the armed forces have not been of the same magnitude. One of the most effective programs resulted in the prevention of nine out of ten attacks of scarlet fever, five out of six infections of the upper respiratory tract of sufficient severity to require hospitalization, virtually all meningococcus infections, and was effective in prevention of pneumococcus pneumonia and gonorrhea. The effect of mass chemoprophylaxis on the incidence of rheumatic fever differs from the effects on meningococcus infections and scarlet fever. It appears that during the first week of a program when the incidence of scarlet fever and meningococcus infections fall precipitously, the incidence of rheumatic fever may actually be increased, but after the first week reduction begins and, if the program is continued for a month or more, the results are impressively good.

The benefits to be derived from mass chemoprophylaxis in any group are related to the need for protection, thoroughness with which the program is carried out, and the care exercised in the prevention of untoward reaction. The decision to give protective drugs to a group of well people must be based upon an estimate that there is a great probability that these now healthy individuals will acquire a preventable disease during a limited period in the near future unless protection is provided. The estimate of danger to the group as a whole is based upon what is known to have recently happened to some members of the group. Prophecy of this sort is a function of the clinical epidemi-

ologist who is guided not by an inner light, but by quantitative data. Discussion of the indications for mass chemoprophylaxis is not within the scope of this paper. It will be dealt with authoritatively, and on the basis of his own brilliant work, by Doctor Coburn.

Forms of Streptococcus Disease. The problems of diagnosis of streptococcus disease are such as to seriously complicate measures for their control. Streptococci cause certain highly characteristic syndromes such as acute tonsillitis, sinusitis, scarlet fever, peritonsillar abscess, acute otitis media, and pneumonia with empyema. A matter of the greatest importance in military and civil practice is the role of streptococci in certain nondescript forms of infection of the respiratory tract—nasopharyngitis, particularly. One of the most impressive effects of mass chemoprophylaxis in military personnel has been the remarkable reduction in incidence of disease of the upper respiratory tract severe enough to require hospitalization. It is not possible to say definitely at present what part the streptococcus plays in this group. It is generally believed that in the great majority of instances streptococcus disease of the respiratory tract is secondary to some virus infection, such as common cold, measles, or influenza. It is encouraging to note in passing that during December 1943, there was an epidemic of influenza in military personnel. Although apprehension was felt, this outbreak was accompanied by relatively low incidence of severe forms of streptococcus disease. This influenza epidemic occurred, fortunately, at a season when streptococcus disease is relatively infrequent and is usually mild. However this may be, in the light of Army experience in World War I, it was very comforting to have available in this epidemic of December 1943 chemotherapeutic agents which are effective against the streptococcus.

Cost of Mass Chemoprophylaxis. The financial expense of furnishing the drug for programs of mass chemoprophylaxis is insignificant. The cost in terms of untoward reactions is a matter of direct concern and responsibility to the clinician. It is the responsibility of the physician to weigh the probable benefits of chemoprophylaxis against the hazards of untoward reactions. From the experience of mass chemoprophylaxis in the armed forces to date, it is clear that the hazards of untoward reactions can be kept to an insignificant minimum; but if civilian and military physicians do not familiarize themselves thoroughly with the peculiar dangers of the combination of prophylactic and therapeutic

use of sulfonamides; if they are careless in the use of this information, disastrous intoxication will occur which may discredit this valuable procedure of preventive medicine and bring down justified criticism of the medical profession. Harmful reactions to prophylactic doses only, do not include renal complications due to crystalluria—the problem is entirely one of sensitivity. Evidences of a sensitivity reaction may be expected in from four to five individuals out of each 1000. These are mild reactions and are of little importance if the drug is stopped soon after their appearance. Severe reactions which are dangerous to the life of the individual may occur in one out of 100,000 individuals from prophylactic doses only. Most sensitivity reactions appear during the second week of the program. The appearance of fever in from two to four hours after a dose of sulfonamide is one of the common manifestations of sensitivity. Unfortunately, thermometry does not lend itself to routine use in large groups of military personnel on a duty status. With few exceptions, the first distinctive evidence of sensitivity reaction is the appearance of skin eruption. In 95 per cent of patients showing skin rash the lesions may be described as morbilliform, erythematous, urticarial, or papular. The rare forms of skin lesions are those of photosensitization, fixed eruption, exfoliative dermatitis, and purpura.

Therapeutic Disasters. A large percentage of severe sensitivity reactions which have developed in those receiving chemoprophylaxis have followed the administration of therapeutic doses of sulfonamides when manifestations of sensitivity to the drug were diagnosed as conditions for which sulfonamide therapy was believed indicated. These mistakes have been mainly as follows:

1. Fever and chills due to sensitivity, but assumed to be caused by pneumonia.

2. Scarlatiniform rash diagnosed as scarlet fever. Sensitivity rash can nearly always be differentiated from scarlet fever by clinical features alone.

3. Tonsillar or pharyngeal lesions diagnosed as acute streptococcus infection, when actually a manifestation of agranulocytosis.

Transfusion of individuals because of the presence of agranulocytosis resulting from sulfonamide sensitivity, with blood from a donor receiving chemoprophylactic doses of sulfadiazine, has led to reactions of extreme severity. The disastrous consequences of therapy with sulfonamides of the sort described above can be eliminated only when

physicians acquire the fixed habit of never prescribing sulfonamides in therapeutic doses until they have satisfied themselves that the condition under treatment does not represent, even in part, a manifestation of sensitivity to sulfonamides. In every instance fever and skin rash should be first suspected as due to sulfonamides.

It is the responsibility of the medical profession, civilian and military, for complete elimination of this group of severe reactions. The responsibility of civilian physicians must be emphasized, because some of the severe reactions have been precipitated by therapy prescribed by civilian physicians to military personnel while on furlough.

The following suggestions are offered as safeguards against serious reaction:

1. Thorough education of medical personnel as to the nature of sensitivity reactions to sulfonamides, and the unique dangers of therapy which arise from programs of prevention.

2. Each individual before beginning chemoprophylaxis should be called upon to report any untoward reaction to previous sulfonamide therapy.

3. Responsibility should be placed upon the individual for reporting the appearance of rash. Individuals who report subjective symptoms which follow the administration of the drug, such as flushing of the skin, feverishness, and pruritus, should be checked by thermometry.

4. Medical supervision should be adequate for early diagnosis of sensitivity reaction and elimination of the individual from the program.

5. Determination of the white blood count whenever there is any basis for suspicion of agranulocytosis and, in every case, before the administration of therapeutic doses of sulfonamide.

6. If penicillin is available, therapy with sulfonamides should be absolutely contraindicated for any individual known or suspected to have recently received sulfonamide for chemoprophylaxis if he manifests rash or leukopenia.

Prophylaxis for the Individual. Information is not yet available which permits evaluation of the extent of protection afforded to the individual in programs of mass chemoprophylaxis by the drug which he himself takes, and the protection due to the fact that others in his environment are also receiving the drug. It is entirely possible that a larger dose would be necessary to protect a single individual in a given epidemiologic situation than if all individuals in the group received the

drug. Furthermore, we must bear in mind that the studies on military personnel dealt with a highly selected group of young men within a narrow age range with a state of physical fitness far beyond that of the average civilian population. When chemoprophylaxis is attempted in individuals in poor general health, and particularly in the older age group, it is not unlikely that greater difficulties may be encountered with toxic manifestations. What principles should guide the practicing physician in the use of chemoprophylaxis for individual patients? We would suggest that two considerations are of importance in evaluating the need of individuals for chemoprophylaxis.

1. Membership in a group in which there is an epidemic and when mass chemotherapy or chemoprophylaxis is not available. No doubt there are situations in civilian groups that resemble, in many important respects, the epidemic conditions of military life.

2. We should consider the possibility of giving protection to those individuals who are prone to frequent severe disabling respiratory infections. For example, individuals with sinusitis, bronchitis or bronchiectasis are commonly asymptomatic until a common cold ushers in a severe incapacitating illness.

The greatest opportunity and responsibility for the use of chemoprophylaxis in individuals is for those who have had attacks of rheumatic fever. The evidence is clear that crippling heart disease from rheumatic fever is, in the main, the result of repeated attacks of rheumatic fever and repeated attacks can be prevented by chemoprophylaxis. The optimum dose for individuals has not been established, but 1 gram daily of sulfadiazine is worthy of extensive trial. Close medical supervision is of the greatest importance during the first four weeks of chemoprophylaxis, with a check in leukocyte count and for febrile or skin reactions at least twice a week during this period. If sensitivity reactions do not appear during the period, it is unlikely that they will occur as long as the drug is taken continuously. The desirability of continuous year-round chemoprophylaxis in such individuals is based on three considerations.

1. Taking the sulfadiazine should become a rigid habit, unbroken by a summer lay-off.

2. The dangers of sensitivity reaction are greatly increased by intermittent use of sulfa drugs.

3. Although streptococcus disease of great severity is rare during

the summer months in most communities, mild infections sufficient to reactivate a rheumatic process are not uncommon. The use of chemoprophylaxis in individuals who have had rheumatic fever is obviously not for use by the impatient physician who demands a maximum of results in the shortest time. It requires the greatest understanding of the personal problems of the individual, and the greatest capacity to persuade the individual to coöperation and self-discipline over a long period of time without arousing such anxiety as to counterbalance the good effects to be obtained.

Summary. We may say that chemoprophylaxis is a new and not fully evaluated tool of preventive medicine, but is proven to have great potentialities for good when properly used in groups or for individual patients. This new technique for use of drugs offers new opportunities and forces new responsibilities on the medical profession. The use of chemoprophylaxis in military medicine requires that a physician try to master the art of diagnosis and prognosis of infections as applied to groups so that it may be prescribed wisely. The most direct and immediate responsibility is for minimizing untoward reactions. This responsibility can be met when every doctor, civil and military, familiarizes himself with the clinical manifestations of sensitivity to sulfonamides, when he recognizes potential disaster which may result from mistaking the manifestations of sensitivity to sulfonamides for conditions which may be benefited by therapy therewith. It is necessary that doctors assume a persistent attitude of intelligent suspicion that clinical manifestations formerly considered innocuous may now be warnings that the patient is susceptible to the greatest harm by the best-intentioned use of an extremely valuable drug.

REFERENCES

1. Thomas, C. B. and France, R. Preliminary report of prophylactic use of sulfanilamide in patients susceptible to rheumatic fever, *Bull. Johns Hopkins Hosp.*, 1939, *64*:67.
2. Coburn, A. F. and Moore, L. V. Prophylactic use of sulfanilamide in rheumatic subjects, *M. Clin. North America*, 1940, *24*:633.
3. Kuhns, D. M. *et al.* Prophylactic value of sulfadiazine in the control of meningococcal meningitis, *J.A.M.A.*, 1943, *123*:335.
4. Watson, R. F., Schwentker, F. F. *et al.* Sulfadiazine in an epidemic of scarlet fever, *J.A.M.A.*, 1943, *122*:730.
5. Coburn, A. F. The prevention of respiratory tract bacterial infections, *J.A.M.A.*, 1944, *126*:88.
6. Holbrook, W. P. The Army Air Forces rheumatic fever control program, *J.A.M.A.*, 1944, *126*:84.

ALLOXAN DIABETES
ITS PRODUCTION AND MECHANISM*

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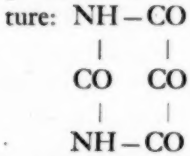
ALLOXAN DIABETES is a new type of experimental diabetes. It is a chemical diabetes caused by treatment with an organic compound, which is related to physiological body substances. It can be produced in the intact animal and in many species. Its signs and symptoms are identical in many respects with those of human diabetes. It is the purpose of this paper to describe the production and the patho-physiology of this diabetes and to compare its mechanism with that of the various other types of experimental diabetes.

Alloxan has been known for more than 100 years, since Woehler produced it by oxidation of uric acid. It has the following structure:



is a colorless powder, melting at 256 degrees centigrade, and is easily soluble in water and alcohol. It decomposes on hydrolysis into urea and mesoxalic acid. Alloxan reduces to dialuric acid with which it acts as an oxidation-reduction system, and its structure is found as a constituent part of the flavine molecule. It has been found to produce profound disturbances in sulfur metabolism, to enhance the endogenous metabolism of liver suspensions, and to be a capillary poison. In spite of this, little evidence exists so far that alloxan plays a role in physiological processes. Uric acid metabolism, so far as is known, does not seem to involve alloxan even as an intermediary product.

The discovery of the diabetogenic action of alloxan is accidental

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as many other great discoveries in science. It is the great merit of the English pathologist Shaw Dunn, whose recent death put an untimely end to a distinguished career, to have observed that alloxan had a specific necrosing action on the cells of the islets of Langerhans. Working on the crush syndrome and the effect of ureides on the kidney, he and his co-workers made this "side observation" and called attention to its importance. Six years earlier, in 1937, Jacobs[1] had found that the injection of alloxan into rabbits resulted in an initial hyperglycemia which was soon succeeded by a severe hypoglycemia, lasting up to eight hours or longer, and terminating with death of the animals unless glucose was administered. All his experiments, however, were acute, and no histological studies were done. Dunn, Sheehan and McLetchie,[2] noticed again this fluctuation of blood sugar in alloxan-treated animals, recognized its relationship to the accompanying anatomical islet cell damage and though their rabbits had all died in a few days, suggested that a sustained diabetes should be the final result of alloxan poisoning.

Their report stimulated work by other investigators, and in only a few months, alloxan diabetes was demonstrated in various animal species. In July, 1943 the production of alloxan diabetes in dogs, and of transitory hyperglycemia and glycosuria in rabbits was announced from our laboratory.[3] Bailey and Bailey reported on alloxan diabetes in rabbits,[4] and Shaw Dunn and his group[5] produced alloxan diabetes in the rat. In the meantime, confirmations and further contributions have been made by several workers in this country and abroad.[6-9]

The diabetogenic doses:

In our laboratory the diabetogenic effect of alloxan has been studied in the dog, the rat, the cat, the rabbit, the rhesus monkey, the pigeon, and the guinea pig.[10-14]

Alloxan diabetes can be produced with one single injection of alloxan. We use freshly prepared unneutralized 5 per cent solution, and give it intravenously or intraperitoneally because of its decided acidity. Neutralization inactivates alloxan, and it decomposes on standing. Scattered small doses and subcutaneous injections have been used also with success.

We define the diabetogenic dose as the amount of alloxan which in eighty per cent of the animals of a given species will produce sus-

tained hyperglycemia and necrosis of the pancreatic islet cells, but which will not cause observable damage to other organs.

TABLE I

Diabetogenic Doses of Alloxan in Various Species

Rat	200-300	mg/kg i. p.
Rabbit	100-200	mg/kg i. v.
Cat	150	mg/kg i. v.
Monkey	100-150	mg/kg i. v.
Dog (dalmatian hound)	50-100	mg/kg i. v.
Pigeon	125-200	mg/kg i. v.

Table I shows the doses as we have found them for various species. It must be mentioned, however, that the response of the cat is very erratic and that of the pigeon develops other metabolic changes frequently, which will be discussed later.

The greatest sensitivity and smallest required dose are found in dogs. Next in sensitivity are monkeys, pigeons and cats; rats and rabbits require the largest dose. The carbohydrate metabolism of the dalmatian hound and the pigeon, which, like man, do not convert uric acid into allantoin, responds in the same way as the animals do which produce allantoin from uric acid.

If a dose larger than the diabetogenic dose is given, severe kidney damage is produced also, and anuria and nitrogen retention develop, and the animals succumb in a few days in a uremic-diabetic syndrome. Still larger doses are fatal within a few hours, probably because of the effect of alloxan on the circulatory system; there is evidence of acute pulmonary edema. The margin between the fatal, the uremic and the diabetogenic dose is the widest in rabbit, rat and dog, smaller in the monkey, and smallest in pigeon, cat and guinea pig. In the latter, no exact diabetogenic dose could be established as yet, since degenerative lesions in the pancreas were found only in those animals which died within 24 hours after injection of alloxan.

The clinical course:

Typical diabetes mellitus will develop about 24 to 28 hours after the injection of a diabetogenic dose of alloxan. The classical signs and symptoms of hyperglycemia, glycosuria, polyuria, and polydipsia will

be present, and very frequently polyphagia and loss of weight. In some species early ketonuria can be observed.

During the first day after the injection, careful observation and supervision are necessary in rabbits and monkeys, lest they die in hypoglycemic convulsions. Frequent administration of glucose may be required. Dogs, rats, and pigeons show less marked initial fluctuations of the blood sugar, and may survive without protection by glucose.

Dogs with alloxan diabetes appear well for about two to three weeks. Their blood sugar remains at a level between 200 and 300 mg. per 100 cc., and their glycosuria may reach four to seven per cent. They eat with great appetite and lose little weight during this period. Glucose tolerance tests show a typical diabetic curve, and insulin sensitivity tests prove that the animals are sensitive to insulin from the very beginning of their diabetes.

Under insulin treatment, the dogs remain in good condition for many months. About one unit protamine zinc insulin per kilo per day is necessary to prevent loss of weight, to maintain normal fasting blood sugar, and to keep the urine free or almost free of sugar. If the dogs remain untreated for more than two or three weeks, they stop eating, lose weight rapidly, appear weak, listless and drowsy, and are very susceptible to infections. This condition is accompanied by the development of hyperlipemia and fatty degeneration of the liver, a phenomenon which may be of importance for the problem of lipocaic, since—as will be shown later—the alpha cells remain intact in alloxan diabetes. The diabetic symptoms very seldom show spontaneous improvement, and usually persist until death occurs in complete emanciation at six to seven weeks.

Monkeys have a very high blood sugar and marked glycosuria following the initial hypoglycemia; ketonuria is present very early. The monkeys look sick and depressed, become emaciated rapidly, and go into acidosis. This is remarkable since monkeys who have been pancreatectomized do not always develop severe diabetes.

Rats, too, show a rapidly progressing diabetes and die in ketosis and acidosis in six to nine days if untreated. Out of a series of 27 rats, which we injected with alloxan, only four did not develop diabetes. All the others showed marked glycosuria and hyperglycemia within 24 to 48 hours. The blood sugar reached values of 300 to 500 mgm. per cent. On the third or fourth day, ketonuria appeared in those animals whose

blood sugar was higher than 300 mgm. per cent. Most impressive was the polyuria: the daily urinary output often exceeded 10 per cent of the body weight. The weight loss averaged 30 per cent in six days. Insulin treatment controls this alloxan diabetes.

The alloxan diabetes of the rabbit also responds to insulin therapy, but its course in the untreated rabbit may take two entirely different forms.

Some animals react, as do dogs, rats and monkeys, and lose weight rapidly, become seriously emaciated, and die within a week. Their blood sugar level is between 400 and 500 mg. per cent, and there is a 5 to 10 per cent glycosuria.

Another group of rabbits respond with similar levels of blood and urine glucose, but after an initial slight weight loss seem to recuperate and regain their weight, appearing in excellent health. Such animals survive without insulin treatment for months, despite the severe diabetes and are apparently capable of compensating for the loss of glucose in the urine by increasing their food intake.

A few rabbits may give the impression of clinical cure of the diabetic condition, the blood sugar returns to normal, the urine is sugar free, but a persistently decreased glucose tolerance test remains to indicate the diabetes.

Of great interest is the development of systemic changes usually considered secondary to diabetes. The great susceptibility to infections has been mentioned before. Of greater importance is the development of cataract which has been observed first by Bailey[15] and of retinitis which was reported by Lewis. It seems, however, that other factors besides the diabetes may contribute to these eye complications. They are not found in all alloxan diabetic animals, and have not yet been observed in our laboratory. So far we have no explanation for this surprising fact.

Pigeons which after total pancreatectomy usually do not develop hyperglycemia and survive indefinitely, respond to alloxan injection with an elevation of the blood sugar to 400 or 500 mg. per 100 cc. They appear sleepy, weak and drowsy, and sit with their eyes closed, making no attempt to move or fly, and die in this stupor. Death, however, may not be due to the diabetes alone; many of the animals develop a very interesting second condition, which has been known for a long time as visceral gout. The blood uric acid content shows a marked

increase to 100 times the initial level and at autopsy, the pericardium and other serous membranes are found to be covered with sodium urate crystals; the kidneys are infiltrated with the same material.

The histological changes:

Only a few remarks on the histological changes after alloxan treatment, which will be dealt with in detail in the following paper by G. Gomori. The pancreatic changes are practically identical in all species mentioned and are characterized by a selective necrosis of the beta cells, as has been shown first in our laboratory, not of the whole islet system. Whereas the beta cells undergo rapid degeneration and disappear finally completely, the alpha cells remain undamaged, at least retain their normal staining property. The beta cell necrosis proceeds without any sign of inflammatory reaction. In dogs the pancreatic ducts usually show a characteristic vacuolization. Of extra-pancreatic changes, the glycogen deposition in the kidney, accompanying pronounced glycosuria, and the necrosis of the convoluted tubules are of importance, the latter occurring only if amounts larger than the diabetogenic doses have been given. The fatty infiltration of the liver, which is most marked in the dog, has been mentioned before. How rapidly the islet cell necrosing action of alloxan starts and how quickly alloxan is made innocuous or removed from the blood stream could be demonstrated recently in the following, not yet published, experiment. We deprived part of the pancreas of its blood supply by means of a clamp, which was released five minutes after the injection of alloxan. Biopsies were taken 24 hours later from both parts of the pancreas and showed that the islet cells appeared essentially normal where the circulation had been interrupted for five minutes only, and that the rest of the pancreas which had had normal blood supply had undergone the typical beta cell degeneration.

The mechanism:

If the degeneration of the beta cells leads to deficient insulin production and thus to diabetes, bioassays of the pancreatic tissue should reveal changes in the insulin content. Assays were carried out on the pancreas of three dogs with alloxan diabetes of a duration of 18, 30, and 60 days. In all three, the insulin content of the pancreatic tissue was markedly decreased. Whereas the pancreas of a normal dog con-

tains about 2 to 3 units of insulin per gram, only ¼ of this amount was recovered from the alloxan-treated organs. This seems to be conclusive evidence that alloxan diabetes is a true pancreatic diabetes and is the result of insulin deficiency. This experiment has found confirmation recently in the work of Ridout, Ham and Wrenshall.[16]

How does alloxan exert its effect upon the islet cells?

Let us recall the early blood sugar fluctuation after alloxan: first a short-lasting hyperglycemia, then a transitory hypoglycemia which in the rabbit frequently requires treatment with glucose, and finally the sustained diabetic hyperglycemia. What is the cause and the significance of these three phases? We shall discuss first the hyperglycemia as a whole, then the initial hyperglycemic phase alone, and finally the hypoglycemia. It is known from the surgical diabetes after partial pancreatectomy, as well as from anterior pituitary diabetes that the initial hyperglycemia precedes the islet cell degeneration and that diabetes will not develop if the hyperglycemia is prevented by starvation, insulin treatment or phlorhizin injection.[17] Here the hyperglycemia is a causal factor of the ensuing diabetes.

In alloxan diabetes such protection is not possible. The diabetogenic process will take its course regardless of whether the initial hyperglycemia is prevented or not. Alloxan affects the islets cells directly.

In a series of experiments which can only be summarized here, we have been able to show that treatment with phlorhizin as well as treatment with insulin will prevent the initial alloxan hyperglycemia, but in spite of the normal blood sugar level the islet cell degeneration will develop. Two dogs were treated for a period of ten days with phlorhizin; on the eighth day they received a diabetogenic dose of alloxan. Diabetes developed as if no phlorhizin had been given. Another dog was given insulin together with and four days following the injection of alloxan. As soon as insulin treatment was discontinued, a marked hyperglycemia and glycosuria became evident. Diabetes had developed as if no insulin had been given. Insulin treatment was renewed after a few days. The same dose was sufficient for diabetic control; there was no change in the sensitivity to insulin. When after a week insulin was stopped, the diabetes became evident again, and a biopsy of the pancreas revealed islet cell degeneration to the same extent as in animals not treated with insulin at all.

Now the early hyperglycemic phase: If insulin is injected together

with alloxan—either as a mixture into the same vein, or separately into different veins—no initial hyperglycemia will develop in the rabbit. The hypoglycemic phase, however, is of the usual severity, and diabetes results as if alloxan alone had been given. The fact that insulin prevents the initial hyperglycemia argues against the possibility that the mechanism is one of insulin inhibition. This possibility was ruled out further when we found that insulin was not inactivated by alloxan in vitro. It seems, therefore, likely that the initial hyperglycemia is not due to lack of insulin, but to mobilization of extra glucose. Such glycogenolysis might be produced by the liver under the influence of epinephrine.[18] As a matter of fact, Young and his associates[9] were able to reproduce the initial alloxan blood sugar curve by the injection of adrenalin and insulin into normal rabbits. We subjected this hypothesis to experimental test by the injection of alloxan into functionally or anatomically adrenalectomized rabbits. The adrenals were extirpated surgically or the medulla was destroyed by intramedullary injection of formalin. A diabetogenic dose of alloxan was given immediately after the removal of the second adrenal or two days after the formalin injection. In no instance did any marked initial hyperglycemia develop. All animals went into severe hypoglycemia and in the few surviving animals diabetes developed. It can be concluded from this experiment that adrenal stimulation of gluconeogenesis is involved in the production of the initial alloxan hyperglycemia. This conclusion is supported by the evidence of histological changes in the adrenal medulla immediately after alloxan injection given by Hard and Carr.[8]

The second phase of the blood sugar reaction to alloxan, the hypoglycemia, was originally interpreted as a result of an insulin-like effect of alloxan itself. Were this true, the blood sugar of depancreatized animals should fall in response to alloxan. We gave diabetogenic doses of alloxan to depancreatized dogs and also to rabbits which previously had been rendered diabetic by alloxan. No lowering of the blood level could be noted in either group. The presence of a normally functioning pancreas, therefore, seems to be necessary for the occurrence of the hypoglycemic reaction, and it would appear that alloxan changes glucose metabolism through its effect on the pancreatic islet cells. This conclusion is further substantiated as well by the work of Corkill, Fantl and Nelson,[7] who found that alloxan did not influence the blood sugar level of the eviscerated cat, and by Ridout and his co-workers

who worked on depancreatized dogs.[16]

If the hypoglycemia is brought about by the effect of alloxan upon the pancreas, it may be caused either by islet cell stimulation or by the release of stored insulin from the degenerating cells. The first view, put forth by Dunn and his co-workers,[2, 5] suggests that increased islet cell activity and overproduction of insulin may overstrain the cells and cause their subsequent failure and degeneration. The experimental evidence, however, seems to favor the second possibility, and we believe with Young and his co-workers[9] that the hypoglycemia is the first sign, rather than the cause, of the developing necrosis. We base our belief on the following evidence:

1. Beta cell degeneration precedes the hypoglycemia. In histological studies on rabbits and dogs we have found that beginning degeneration of the beta cells is demonstrable as early as one hour after injection with alloxan. It precedes the hypoglycemia which usually develops only after four to five hours.

2. It is impossible to separate the hypoglycemic effect from the diabetogenic action. Whenever hypoglycemia develops, diabetes follows if the animal survives.

The specificity of alloxan:

A few words must be said about the remarkable specificity of alloxan as a diabetogenic agent. Dunn and his co-workers[2] had found that not only alloxan but also a quinoline compound (styryl-quinoline No. 90) produced islet cell necrosis in rabbits. They tested several other compounds as oxalic acid, uranium, quanidine and uric acid, and found that none of them had a diabetogenic or islet cell necrosing effect. Earlier Jacobs[1, 10] had found that none of a series of more than sixty chemical compounds produced the fluctuation of the blood sugar level which is characteristic for early response to alloxan. No alloxan-like action was found in a series of compounds tested by Thorogood.[20] We have tested three groups of substances: compounds which are chemically related to alloxan, such as dialuric acid, the reduction product of alloxan, and alloxantin, which is formed by interaction of alloxan and dialuric acid; oxidizing agents, which were tested on the assumption that alloxan may owe its effect to its oxidizing property, and quinoline and cinchophen as relatives of styryl-quinoline, which is not available on the market. These substances were injected in various doses in dogs

and the blood sugar level as well as the histological appearance of the pancreas were followed. None of them exhibited any diabetogenic activity though several of them proved to be very toxic. The specificity of the diabetogenic action of alloxan, therefore, remains unexplained. It seems, however, unlikely that its oxidizing property is the diabetogenic factor.

Comparison with other forms of experimental diabetes:

In a comparison of alloxan diabetes with other forms of experimental diabetes it must be pointed out once more that the diabetes of alloxan poisoning is the result of *direct* damage to the beta cells. This chemical diabetes thus differs significantly from the surgical diabetes of partial pancreatectomy and from the endocrine diabetes of treatment with anterior pituitary extract (APE diabetes)[21,22] though all three have in common the degeneration of the beta cells. This difference can be summarized as follows:

1. Both surgical and APE diabetes are considered to be due to overwork of the beta cells since they can be prevented by starvation or by insulin in the early stages of the disease. Alloxan diabetes is the result of direct action upon the islet cells and cannot be prevented either by starvation or by insulin.

2. The pituitary extract has to be administered in increasing doses over a period of several days, while alloxan is effective in a single dose.

3. Some species, as for instance, rats, can be made diabetic by APE only after extensive resection of the pancreas, while alloxan is effective in the intact animal.

4. APE diabetes in its early stage is insulin-resistant while alloxan diabetes is not.

5. The histologic changes in APE diabetes are mitoses, degranulation and vacuolization of the beta cells with subsequent atrophy and hyaline changes. In alloxan diabetes the changes are those of an irreversible acute degeneration, without vacuolization or fibrosis.

It must be emphasized further that alloxan proved to be diabetogenic even in those species in which total pancreatectomy is not always followed by severe diabetes.

What the significance of alloxan diabetes may be for the pathogenesis of human diabetes must be left for further investigation. Whatever the outcome may be, it can be stated already that we have found

in the diabetogenic action of alloxan not only new means to study disturbed carbohydrate metabolism but also a previously unknown mechanism which causes beta cell degeneration and diabetes through the toxic effect of an organic compound related to protein metabolism.

Therapeutic implications:

Finally, attention should be called to a clinical implication. Brunschwig[23,24] has suggested that the islet cell-necrosing property of alloxan might be of use in the treatment of hyperinsulism and islet cell tumors. He gave alloxan to a few patients with inoperable carcinoma and to one patient with metastatic islet cell tumor. The clinical results which we observed with him were not very striking, and the histologic changes in the islet cells were surprisingly slight. The observations, however, are too limited to permit conclusions as to the advisability and effectivity of such treatment. The fact that many animal species have been found sensitive to the islet cell-necrosing action of alloxan makes it likely that man will be no exception. In any case, the remarkable—and almost unique—fact of the specific action of a chemical compound on a highly differentiated specific cell system deserves greatest interest and thorough examination.

REFERENCES

1. Jacobs, H. R. Hypoglycemic action of alloxan, *Proc. Soc. Exper. Biol. & Med.*, 1937, *37*:407.
2. Dunn, J. S., Sheehan, H. L. and McLetchie, N. G. B. Necrosis of islets of Langerhans produced experimentally, *Lancet*, 1943, *1*:484.
   Dunn, J. S., Kirkpatrick, J., McLetchie, N. G. B. and Talfer, S. V. Necrosis of islands of Langerhans produced experimentally, *J. Path. & Bact.*, 1943, *55*:245.
3. Brunschwig, A., Allen, J. G., Goldner, M. G. and Gomori, G. Alloxan, *J.A.M.A.*, 1943, *122*:966.
4. Bailey, C. C. and Bailey, O. T. Production of diabetes mellitus in rabbits with alloxan; preliminary report, *J.A.M.A.*, 1943, *122*:1185.
5. Dunn, J. S. and McLetchie, N. G. B. Experimental alloxan diabetes in the rat, *Lancet*, 1943, *2*:384.
6. Carrasco-Formiguera, R. Alloxan diabetes in dogs, *J. Lab. & Clin. Med.*, 1944, *29*:510.
7. Corkill, A. B., Fantl, P. and Nelson, J. F. Experimental diabetes, *M. J. Australia*, 1944, *31*, pt 1:285.
8. Hard, R. L. and Carr, C. J. Experimental diabetes produced by alloxan; *Proc. Soc. Exper. Biol. & Med.*, 1944, *55*:214.
9. Hughes, H., Ware, L. L. and Young, F. G. Diabetogenic action of alloxan, *Lancet*, 1944, *1*:148.
10. Goldner, M. G. and Gomori, G. Alloxan diabetes in the dog, *Endocrinology*, 1943, *33*:297.
11. Gomori, G. and Goldner, M. G. Production of diabetes mellitus in rats with alloxan, *Proc. Soc. Exper. Biol. & Med.*, 1943, *54*:287.
12. Goldner, M. G. and Gomori, G. Mech-

anism of the diabetogenic action of alloxan, *Proc. Soc. Exper. Biol. & Med.*, 1944, *55*:73.

13. Goldner, M. G. and Gomori, G. Studies on the mechanism of alloxan diabetes, *Endocrinology*, 1944, *35*:241.
14. Goldner, M. G. and Gomori, G. Alloxan diabetes, *Proc. Am. Diabetes A.*, 1944, *in press.*
15. Bailey, C. C., Bailey, O. T. and Leech, R. S. Alloxan diabetes with diabetic complications, *New England J. Med.*, 1944, *230*:533.
16. Ridout, J. H., Ham, A. W., Wrenshall, G. A. Correlation of the insulin content and the histological picture of the pancreas at intervals after the administration of insulin, *Science*, 1944, *100*:57.
17. Lukens, F. D. W., Dohan, F. C. and Wolcott, M. W. Pituitary-diabetes in the cat; recovery following phlorhizin treatment, *Endocrinology*, 1943, *32*:475.
18. Campbell, J., Haist, R. E., Ham, A. W. and Best, C. H. On the mechanism of epinephrine hyperglycemia, *Am. J. Physiol.*, 1940, *129*:328.
19. Jacobs, H. R., *personal communication.*
20. Thorogood, E. Alloxan-induced diabetes in rats, *Federation Proc.*, 1944, *3*:48.
21. Houssay, B. A., Foglia, V. G., Smyth, F. S., Rietti, C. T. and Houssay, A. B. Hypophysis and secretion of insulin, *J. Exper. Med.*, 1942, *75*:547.
22. Young, F. G. Anterior pituitary gland and diabetes mellitus, *New England J. Med.*, 1939, *221*:635.
23. Brunschwig, A. and Allen, J. G. Specific injurious action of alloxan upon pancreatic islet cells and convoluted tubules of the kidney; attempted chemotherapy of insulin-producing islet cell carcinoma in man, *Cancer Research*, 1944, *4*:45.
24. Brunschwig, A., Allen, J. G., Owens, F. M. and Thornton, T. F. Alloxan in the treatment of insulin producing islet cell carcinoma of the pancreas, *J.A.M.A.*, 1944, *124*:212.

Editor's Note

A related paper by Dr. George Gomori, Department of Medicine, The University of Chicago, entitled: "The Histology of the Normal and Diseased Pancreas," which was presented before the New York Diabetes Association, September 28, 1944, will appear in the February issue of the Bulletin together with a discussion of these two papers by Dr. Paul Klemperer, Pathologist, The Mt. Sinai Hospital, New York.

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